D E C L A R A T I O N

I, SHINICHI USUI, a Japanese Patent Attorney registered No.9694, of Okabe International Patent Office at No. 602, Fuji Bldg., 2-3, Marunouchi 3-chome, Chiyoda-ku, Tokyo, Japan, hereby declare that I have a thorough knowledge of Japanese and English languages, and that the attached pages contain a correct translation into English of the priority documents of Japanese Patent Application No.2003-209247 filed on August 28, 2003 in the name of CANON KABUSHIKI KAISHA.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

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SHINICHI USUI

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[Inventor]

[Domicile or Residence] c/o Canon Kabushiki Kaisha
30-2, 3-chome, Shimomaruko, Ohta-ku, Tokyo

[Name] NOBUKO YAMAMOTO

[Applicant]

[Identification No.] 000001007

[Domicile or Residence] 30-2, 3-chome, Shimomaruko, Ohta-ku, Tokyo

[Name] CANON KABUSHIKI KAISHA

[Representative] FUJIO MITARAI

[Telephone Number] 03-3758-2111

[Attorney]

[Identification No.] 100090538

[Domicile or Residence] c/o Canon Kabushiki Kaisha
30-2, 3-chome, Shimomaruko, Ohta-ku, Tokyo

[Patent Attorney]

[Name] KEIZO NISHIYAMA

[Telephone Number] 03-3758-2111

[Elected Attorney]

[Identification No.] 100096965

[Domicile or Residence] c/o Canon Kabushiki Kaisha
30-2, 3-chome, Shimomaruko, Ohta-ku,
Tokyo

[Patent Attorney]

[Name] HIROKAZU UCHIO

[Telephone Number] 03-3758-2111

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[Title of the Invention] Target Substance Quantifying Method and Probe Carrier Used in the Method

[What is Claimed is:]

[Claim 1]

A method of simultaneously quantifying two or more kinds of target substances in a solution, the method comprising the steps of:

preparing a probe carrier in which probes which can be specifically bonded to the target substances are immobilized at known positions on the carrier;

contacting the carrier with the solution to bind the target substance to the probes; and

measuring the amount of the target substance bonded to the probe, wherein

the probes which can be bonded to the target substance of the amount exceeding the amount of each of the target substances in the solution are immobilized onto the probe carrier.

[Claim 2]

The quantification method according to Claim 1, wherein the amount of probes immobilized on the probe carrier is made different respectively, depending on the kind of the probe.

[Claim 3]

The quantification method according to claim 1, wherein the amount of probes immobilized on the probe carrier is fixed, respectively, at 1.0 to 2.0 times as much as the amount expected for the target substance in the solution.

[Claim 4]

The quantification method according to Claim 1, wherein the probe carrier is a tape and the step of contacting comprises the step of contacting part of the probe carrier with the solution and the step of sequentially changing the contact part with the solution by relatively moving the carrier.

[Claim 5]

A probe carrier which is used to simultaneously quantify two or more kinds of target substances in a solution and on which probes capable of specifically binding to a target substance are immobilized at known positions on the carrier, characterized in that the probe which can be specifically bonded to the target substances of the amount exceeding the amount of each of the target substances in the solution is immobilized onto the probe carrier.

[Claim 6]

A gene quantifying carrier which is used to simultaneously quantify two or more kinds of genes in a solution and in which probes which can be specifically bonded to the genes are immobilized at known positions on the carrier, wherein

areas containing the probes are separately provided as spots on the carrier and the number of spots set for two or more kinds of plural genes to be detected is different for different kind of probe.

[Claim 7]

The gene quantifying carrier according to Claim 6, wherein the amount of probe immobilized onto the spot is known.

[Claim 8]

The gene quantifying carrier according to Claim 7, wherein the number of molecules of the probe on each spot is equal among all kinds of probes.

[Claim 9]

The gene quantifying carrier according to Claim 8, wherein the number of molecules of the probe on each spot is on the same order as the minimum number of molecules of an mRNA of a gene to be evaluated existing in a sample.

[Claim 10]

The gene quantifying carrier according to Claim 9, wherein the number of spots in each of the areas is proportional to an average amount of expression, in human, of the target gene having a sequence complimentary to the probe.

[Claim 11]

The gene quantifying carrier according to Claim 7, wherein the number of molecules of the probe on the spot is the same between the probes of the same sequence and the number of molecules varies between probes of different sequences.

[Claim 12]

The gene quantifying carrier according to Claim 6, wherein the spot is formed by printing by an ink jet method.

[Claim 13]

The gene quantifying carrier according to Claim 6, wherein the number of spots in each of the area differ 100 to 1000 times between the maximum and the minimum.

[Claim 14]

A gene quantifying carrier, wherein

probes of each sequence are immobilized on the same carrier as a plurality of spots such that the probes of respective kinds have spots of the same number,

the amount of probes on one spot is known and the number of molecules of the probe is the same between the probes of the same sequence and is different between the probes of different sequences.

[Claim 15]

The gene quantifying carrier according to Claim 14, wherein the spot is formed by printing by an ink jet method.

[Claim 16]

The gene quantifying carrier according to Claim 14, wherein a probe immobilized area on the carrier is divided into sections having the same area and a group of spots to which the probes of the same sequence are immobilized is disposed in each divided section of the area.

[Claim 17]

The gene quantifying carrier according to Claim 14, wherein the carrier is a tape.

[Claim 18]

The gene quantifying carrier according to Claim 14, wherein the carrier is a plate substrate.

[Detailed Description of the Invention]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to a probe carrier used for simultaneous quantification of a plurality of kinds of target substances and a quantification method using the probe carrier. More particularly, it relates to a probe carrier for quantification of expressed genes and a quantification method using the same.

[0002]

[Prior Art]

In recent years, methods of analyzing gene structures have been making remarkable progress. The structures of many genes, including human genes, have been elucidated. For analysis of such genes, DNA chips (DNA microarrays) have come to be used, which are constituted by spotting and immobilizing DNA fragments (hereinafter called "probe DNA") of more than thousands to ten thousands of different kinds in lines on a substrate, such as a microscope slide glass (for example, Citation 1).

Analyses of the amount of gene expression have come to serve for drug discovery, disease prediction,

disease diagnosis, decisions of therapeutic policies and so forth. Likewise, protein chips are also used for quantification of expressed proteins.

[0003]

[Patent Document 1]

Japanese Patent Application Laid-Open No. H11-187900

[0004]

[Problems to be Solved by the Invention]

For quantifying a DNA in a sample solution using the above-mentioned DNA microarray, the amount of fluorescence of a hybrid with labeled sample DNA that binds to this microarray is measured.

[0005]

In conventional arrays, only one probe-binding site was allocated for one gene, and each site contains almost the same amount of immobilized probes.

[0006]

However, the DNA content in a sample varies greatly with genes; in many cases, the high content and the low content differ 100 to 1000 times.

[0007]

Therefore, for carrying out quantification at a plurality of probe-binding sites simultaneously using a solution containing DNAs of which concentration are different about 1000 times, simultaneous measurement is difficult with conventional microarrays of which spots contain probes of almost the same amount. This

is because some probes become short in quantity for target substances present in a large amount, while some are present in an extremely excessive amount for target substances present in a small amount. Moreover, when such an array was used, it is impossible to quantify the amount of fluorescence from each spot in the same dynamic range; the intensity of fluorescence had to be measured for every site individually by changing the dynamic range.

[0008]

The present invention has been made in order to solve the above mentioned problems. Accordingly, one object of the present invention is to provide a method of simultaneously and readily quantifying a plurality of target substances. Another object of the present invention is to provide a gene quantifying carrier realizing quantification in a predetermined measuring range. A further object of the present invention is to provide a method of quantifying a gene on the basis of the amount of signals.

[0009]

[Means for Solving the problems]

As a result of earnest investigation in view of the above mentioned problems, the inventors of the present invention have found that ready and simultaneous quantification of the amount of all

target samples is realized by controlling the amount of probes on an array in accordance with the kind and amount of the sample in a solvent which is in contact a DNA array and then made the present invention on the basis of the above finding.

[0010]

That is, according to the present invention, there is provided a target substance quantifying method comprising the steps of preparing a probe carrier in which probes which can be specifically bonded to the target substances are immobilized at known positions on the carrier, contacting the carrier with the solution to bind the target substance to the probes and measuring the amount of the target substance bonded to the probe, wherein the probes which can be bonded to the target substance of the amount exceeding the amount of each of the target substances in the solution are immobilized onto the probe carrier.

[0011]

According to the present invention, the target substances are bonded using the probe carrier having probes of the amount exceeding that of each target substance of a plurality of kinds of target substances in a solution, so that there is no problem that an excessive state occurs while the step of bonding the respective target substance is being

performed and thus there can be provided the method of simultaneously and readily performing quantitative analysis on a plurality of different kinds of target substances in a solution. Specifically, the probe DNA of the amount exceeding the amount of each DNA is immobilized on a carrier even in a system in which a DNA which is large in expression amount and a DNA which is small in expression amount are mixedly present as in gene expression analysis of a man, so that there can be provided a method of simultaneously performing quantitative analysis on all DNAs.

[0012]

Further, by using a probe carrier onto which the probes of the amount sufficiently corresponding to the amount of a target substance concerned are immobilized, respective hybrids can be controlled to the amount suitable for measurement of the signal intensity, such as measurement of fluorescence, thereby enabling simple and convenient measurement without the need of changing dynamic ranges.

[0013]

In addition, it is also possible to change the intensity of fluorescence to the intensity suited for detection upon fluorescent detection of hybridization reaction by immobilizing the probes of the amount corresponding to the amount of the sample in the solution onto the carrier and disposing the probes in

a state suited for detection.

[0014]

[Preferred Embodiments of the Invention]

Next, a quantifying method of the present invention will be described in detail.

[0015]

The quantifying method in accordance with the present invention is done in the following steps:

(1) The step of preparing a probe carrier in which a probe which can be specifically bonded to the target substance is bonded at a known position on the carrier;

(2) The step of contacting the carrier with the solution to bind the target substance to the probes; and

(3) The step of measuring the amount of the target substance boded to the probe.

[0016]

Next, the respective steps will be described in detail.

[0017]

<Preparation of the probe carrier in which the probe which can be specifically bonded to the target substance is bonded at the known position on the carrier>

First, the step of preparing a probe carrier having probes capable of specifically binding to a

target substance bound to known locations on the carrier will be explained. Exemplary of this step is, for example, fabrication of a DNA microarray and the like. It is typical configurations of DNA array that various DNA probes are adsorbed on the surface of a glass substrate treated suitably for immobilizing DNA by a strong bond, such as covalent bond. Also there are resin substrates and substrates coated with thin metal film.

[0018]

In the present invention, a windable tape-shaped base material may be used. In that case, a method may be adapted of contacting the probe solution with only part of this tape-shaped base material and moving sequentially the position for contacting with the solution, which will be described later.

[0019]

<Target substance and the probe which can be specifically bonded to the target substance>

In the present invention, a probe to be immobilized onto a carrier is capable of specifically binding to a specific target substance. Further, examples of this probe include oligonucleotides and polynucleotides, or other polymers, capable of recognizing a specific target. The term "probe" as used herein refers both to a molecule having a probe

function, such as a polynucleotide molecule, and a population of molecules having the same probe function, such as polynucleotides of the same sequence which have been surface-immobilized at dispersed positions, including a molecule called a ligand. Moreover, a probe and a target are often used interchangeably, and can be bound to the target as part of ligand/anti-ligand (which may also be referred to as a receptor) pair, or may come to bind. For the purpose of the present invention, a probe and a target may include naturally occurring bases or their analogs.

[0020]

One example of a probe supported on a carrier is an oligonucleotide containing a nucleotide sequence hybridizable with a target nucleic acid, which has a binding portion to be linked to the carrier surface via a linker. The position of such a binding portion in the probe oligonucleotide molecule is not limited as long as it does not hinder the desired hybridization reaction.

[0021]

A probe usable for the probe carrier manufactured by the method of the present invention is appropriately selected according to the purpose of use. Preferable probes for the purpose of advantageously embodying the present method are DNA,

RNA, cDNA (complementary DNA), PNA, oligonucleotides, polynucleotides, other nucleic acids, oligopeptides, polypeptides, proteins, enzymes, enzyme substrates, antibodies, epitopes to an antibodies, antigens, hormones, hormones, receptors, ligands, ligand receptors, oligosaccharides and polysaccharides. A combination of two or more thereof may be used as necessary.

[0022]

As used herein, "probe carrier" refers to a carrier having a plurality kinds of probes immobilized onto the carrier surface (including the surface of the inner walls of hollow members or tubular carrier members) as independent areas, such as dot-like spots; and "probe array" refers to a carrier having such probes aligned at a predetermined interval.

[0023]

It is desirable that a probe has a structure capable of binding to the surface of a carrier and that immobilization of a probe onto a carrier be performed via this bindable structure. In that case, preferably, the structure of the probe enabling binding to a carrier surface is formed by the treatment that introduces an organic functional group to the probe, such as amino group, mercapto group, carboxyl group, hydroxyl group, acid halides

(haloformyl group; -COX), halides (-X), aziridine, maleimide group, succinimide group, isothiocyanate group, sulfonyl chloride group ($-SO_2Cl$), aldehyde group (formyl group; -CHO), hydrazine, and acetamide iodide. Further, the surface of the carrier may be subjected to necessary treatment according to the binding structure of the probe.

[0024]

<Disposition and configuration of probes on a carrier>

The probe carrier in accordance with the present invention may include the following configurations.

[0025]

The probe carrier may include a configuration in which an area in which a plurality of probes are immobilized at different known positions on the carrier is present in the form of a spot or in the form of an elongated striped-shape.

[0026]

In order to immobilize more kinds of probes as separated spots, the area of each spot is preferably 10 μm^2 to 500 μm^2.

[0027]

In addition, as for each probe, the following configurations can variably control the amount of probes to be bound to a carrier:

(A) Respective probe-immobilized areas have the same size and the amount of probes per unit area is different for different area.

(B) The amount of probes per unit area in each area is fixed and the sizes of the areas are different.

(C) The size of each area and the amount of probes per unit area of each area are different from area to area.

(D) The carrier is constituted by a plurality of probe-immobilized areas which are constant in the amount of probes per unit area in each area and carriers are the number of probe-immobilized areas is different for different area.

(E) The carrier is constituted by a plurality of probe-immobilized areas which are different from one another in the mount of probes per unit area in each area and the number of probe-immobilized areas is the same as those in other areas .

(F) The carrier is constituted by a plurality of probe-immobilized areas which are different in the amount of probes per unit area in each area and the number of the probe-immobilized areas is different for different area.

[0028]

The respective probe immobilized areas may be disposed either discretely or close to each other.

[0029]

<Method of forming spots>

For the method of forming bonded-probe-formation areas (spots) on a carrier, any conventionally known methods may be used.

[0030]

For example, spots can be formed using an ink jet method or a photolithography technique.

[0031]

Formation of a spot by an ink jet method is performed by ejecting a solution containing a probe as a minute droplet to apply the droplet onto a probe carrier. This method makes it possible not only to have the landing diameters (which practically correspond to the spot diameters) uniform but also to easily change the amount of probes present in a spot area by changing the concentration of the solution to be ejected, whereby the probe carrier of the present invention can be suitably fabricated.

[0032]

Further, using an ink jet method, it is also possible to easily form spots having different landing diameters (spot size) with an ejection solution of a certain probe concentration by appropriately changing the ejection energy etc. In this case, the probe density in a spot (the number of probe molecules/spot area) is the same for each spot;

it is possible to make the average luminance of 80% of probe hybridization almost same for example. This provides a quantification method without the need of changing the dynamic range.

[0033]

<Adjustment of the immobilization amount of probe and amount of target substance>

In the present invention, it is preferable to predict the order of the immobilization amount of the probe on a probe carrier in advance from the sample solution containing a target substance. In some cases, the concentration of the solution to be contacted may be adjusted in the light of the amount of the probe on a probe carrier. However, this cannot adjust respective concentrations of respective target substances. Thus, for quantitative analysis of respective target substances, it is very important to adjust the immobilization amount of the probe on a probe carrier with respect to the amount of a target substance.

[0034]

Of course, strict adjustment of each component amount is not necessarily required. For example, for analyzing expression of two different genes A and B, when it is expected that the expected expression amount of the gene A is nearly 100 times as much as that of the gene B, the probe carrier may have probes

different in the immobilization amount by about 100 fold. To be more specific, when 0.01 μmole mRNA and 10 μmole mRNA expressed from gene A and gene B respectively are present in a solution, a probe carrier may be prepared which has 0.012 μmole of nucleic acid probe A', capable of specifically binding to the mRNA expressed from gene A, and 12 μmole of nucleic acid probe B', capable of specifically binding to the mRNA expressed from gene B, immobilized on the carrier.

[0035]

<Step of contacting a probe carrier with a solution containing a target substance>

Next, the step of contacting a probe carrier with a solution containing a target substance will be explained.

[0036]

In this step, a probe carrier is contacted with a solution containing a target substance. As previously described, the amount of the target substance in the solution is predicted in advance, and then the solution is contacted with the probe carrier, so that the target substance binds to a probe on the probe carrier. When both the target substance and the probe are nucleic acid, nucleic acid hybrids are formed by a hybridization reaction. The conditions under which the hybridization reaction

occurs vary with the nucleic acid to be used, but similar conditions may be used to those for hybridization on an ordinary solid substrate.

[0037]

In addition, when a windable tape-shaped base material (as will be described hereafter) is used, a probe solution is contacted with only part of this tape-shaped base material. After a hybridization reaction of the probe present in the portion in contact with the solution and the target substance in the solution occurs sufficiently, the tape-shaped probe carrier will be moved in relation to the solution such that another part of the probe carrier is contacted with the solution. By repeating this action, the binding between the probes and the target substances is observed. The detailed procedure will be explained later.

[0038]

<Step of measuring the amount of the target substance bonded to the probe>

Next, the step of measuring the amount of the target substance boded to the probe will be described.

[0039]

Examples of this step include a method measuring the amount of a target substance by binding of a fluorescent-labeled target substance to a probe on a probe carrier by fluorometry.

[0040]

For example, the amount of genes is calculated by counting the number of spots emitting signal and adding up the signal intensity of each spot.

[0041]

Instead of measuring the signal intensity per spot, it is also possible to read signal in every row of spots using so-called line sensor. In this case, it is important to arrange one kind of probe in the same row; preferably, the probe for expression in a small quantity and thus should be provided as one spot is present as only one spot in one row.

[0042]

According to another embodiment of the gene quantifying carrier, there is given a method of controlling the density of probes such that the spots of respective probes occupy the same area as shown in Fig. 2. Also, in this case, a configuration that the density of the probes to be spotted in each solution is constant regardless of the kind of the probe used may be adopted.

[0043]

The intensity of the signal per each area corresponds to the amount of expression of respective genes. It is preferable to use an area sensor for measurement in terms of miniaturization and low cost.

[0044]

Considering that the total signal intensities of respective areas are compared, it is possible, as shown in Fig. 3, to provide the same number of spots using solutions of different concentrations for each probe or, alternatively, to fabricate a carrier using different probe concentrations and different spot numbers for respective areas.

[0045]

In some cases, fluorescent measurement may lack reproducibility because the fluorescence of the labeling agent (pigment etc.) may fade by one measurement. The conventional measurement changing the measuring range results in repeated measurement of the same site; causing a problem in reproducibility and in intensity comparison between respective ranges. In the method of the present embodiment, one measurement in the same dynamic range is possible. In addition, a simple and convenient measuring apparatus that does not require range adjustment function and the like can be used.

[0046]

However, in any case, it is preferable to select optimal combination, based on examination of the relationship between the amount of expression and the sensitivity of the sensor.

[0047]

Hereafter, the embodiments of the present

invention will be explained.

[0048]

Embodiment 1

The following describes an embodiment in which the carrier is a flat substrate like a slide glass.

[0049]

In this embodiment, the amount of the immobilized probe on the carrier is larger than that of the corresponding target substance of interest in a sample solution and the amounts of the immobilized probes are different according to corresponding target substances.

[0050]

<Test plate for quantification of biological sample and its use>

The test plate according to the present embodiment comprises a glass substrate of a commercial size on which two or more areas are formed where different probes are disposed respectively.

[0051]

The test plate can be manufactured by attaching a liquid containing a probe to the carrier using application means while moving the means or carrier. For such liquid application means, a liquid ejection apparatus that ejects a liquid from the openings of various pipettes or nozzles can be used.

[0052]

In this embodiment, it is preferable, as shown in Fig. 1, the same probes are immobilized in the width direction and different probe species are disposed in the longitudinal direction of the substrate. In addition, the areas where the same probes are immobilized may be different in size (area). In addition, the densities of the spots may vary with locations.

[0053]

Further, these density variation may be made by the changes in the number of spots applied by ink jet etc.

[0054]

When a mode is employed in which binding reaction between the probe and the substrate occurs on the substrate, the liquid containing the probe is applied to the substrate to form an attachment area. In this state, the probe starts to react with the carrier, whereby a probe immobilization area is formed there. While when a specific treatment for immobilizing the probe to the substrate is required, the substrate on which the probe liquid was attached is subjected to appropriate immobilization treatment.

[0055]

<Carrier for gene quantification and its use>

An embodiment of the probe-carrier for gene quantification is that shown in Fig. 1, where

solutions of various probes are prepared to the same concentration and the spot number is altered to reflect the expression amount of respective target genes in cells, e.g., human tissue, that is, large spot number for a highly expressed gene and small spot number for a gene with reduced expression.

[0056]

In this case, if the number of the molecules of a gene with the lowest expression among the expressed genes in a sample and the number of the probe molecules in one spot are made to agree and the agreed value is used as the unit, quantification of genes that are greatly different in expression becomes possible by setting the number of spots to 100, 1,000 etc., according to the expression degree of the genes.

[0057]

Further, it is preferable that the total amount of one probe immobilized on the aforementioned probe carrier is 1.0 to 2.0 times as much as the amount expected for its target substance in the solution.

[0058]

Several specific examples of a probe carrier having the above-described configuration will be illustrated below.

[0059]

Example 1

Preparation of a plate for quantification of gene expression

A commercially available slide glass was set on an ink jet printer in the same manner as paper. Six kinds of oligonucleotide probe DNAs having different sequences were filled into six ink cartridges of this ink jet printer and printing was carried out on a slide glass as shown in Fig. 1. The printed slide glass was heated to 180°C to immobilize the probe DNA on it.

[0060]

<Analysis of expression amount using a plate for quantification of gene expression>

Reaction between a target substance contained in a sample and a probe immobilized on a carrier can be signalized by various methods. Usually a labeling substance is used, such as a fluorescent substance that can emit optical signal detectable by a sensor and the like.

[0061]

Amplification and fluorescent-labeling of mRNA can be carried out by a conventional in vitro transcription method (e.g., the MegaScript kit, Ambion Ltd.).

[0062]

When such a sample solution is contacted with the probe carrier and then the reaction between the

fluorescent labeled target substance and the probe is detected as fluorescence, the amount of reaction between the probe and the target substance in each probe immobilization area can be determined by moving the probe carrier or the detection means such as a sensor.

[0063]

When using a liquid ejection apparatus to apply a liquid to a substrate such as a probe solution, it is advantageous to use a liquid ejection apparatus that has a plurality of liquid-ejection members each comprising a liquid container, a nozzle for ejecting the liquid connected to the liquid container and means of generating liquid ejection energy for ejecting liquid from the nozzle, of which number may be selected depending on the kinds of liquids to be ejected.

[0064]

Means of generating liquid ejection energy include various methods, such as a piezoelectric method and a heating method. However, in arranging a large number of liquid ejection parts in high density, each of which needs to be provided independently, a heater element capable of generating thermal energy, causing film boiling by heating a liquid and ejecting a liquid from the opening of the nozzle using the pressure may be suitably used.

[0065]

More preferable is a heater element having a structure in which a bubble produced by film boiling communicates with the open air via the opening of the nozzle.

[0066]

Embodiment 2

The following embodiment illustrates the one in which the carrier is composed of a windable tape-shaped material.

[0067]

In this embodiment, probes on a carrier are present in an excessive amount to the target substance of interest in a solution. In this embodiment, instead of contacting the whole probe-bearing carrier simultaneously, only part of the carrier is contacted with the solution sequentially, whereby the amount of the target substance in the solution is estimated.

[0068]

<Test tape for quantification of biological samples and its use>

The test tape for quantification of biological samples in accordance with the present embodiment includes two or more areas on which different probes are disposed in the longitudinal direction of the long substrate.

[0069]

Meanwhile, the test tape of the present embodiment may also be provided in the form of pieces, such as a thread, a string, a belt and a chip. Further, the tape may be wound around a core to be provided as a roll or a bobbin and used continuously in the unwound state, or used as a piece that has been cut in an appropriate length.

[0070]

Moreover, the test tape for quantification of biological samples in accordance with the present embodiment can be manufactured by attaching a liquid to a carrier while moving the probe solution-applying means along the longitudinal direction of the tape. For such means for applying a liquid, a liquid ejection apparatus that ejects a liquid from the openings of various pipettes or nozzles can be used.

[0071]

As shown in Fig. 4, the test tape for quantification of biological samples has the same probes immobilized in its width direction and plural different probe species immobilized in its longitudinal direction. The areas in which different probes are immobilized respectively may have the same area (size) and the spot density may vary depending on the areas.

[0072]

Moreover, as shown in Fig. 5, in one area where the same substance is immobilized, the density of the immobilized substance may vary in the longitudinal direction.

[0073]

What is more, such a density change may be achieved by changing the number of spots, by using an ink jet etc. (Figs. 6 and 7).

[0074]

Such density change can also be automatically made using a gradation function of ink jet printing. Fig. 8 shows an example of a probe carrier on which probe density continuously varies in the longitudinal direction of the carrier.

[0075]

In addition, a tape-shaped carrier includes a string-shaped one as shown Fig. 9.

[0076]

Fig. 10 shows an example of the step of applying a liquid containing a probe while moving a liquid ejection apparatus relatively to a string-shaped substrate. A liquid ejection apparatus 3 has nozzle openings 3a-1 to 3a-n, each of which ejects a liquid containing a different probe, and these nozzles are disposed linearly in the longitudinal direction of the carrier. The nozzle alignment of the liquid ejection apparatus 3 may be as follows: A

plurality of nozzles are disposed in the direction intersecting at right angles to the longitudinal direction of the carrier, and the position of nozzles in that direction can be changed as necessary so that nozzles facing the carrier can be exchanged. This makes it possible to apply many kinds of probe liquids from as many nozzles as possible. In the example shown in Fig. 10, a carrier 1 moves relatively in the direction of the arrow to the liquid ejection apparatus 3. First, a first probe solution is applied to the carrier from the nozzle 3a-1 to form attachment area 2-1. Next, different probe solutions are ejected one by one from nozzles 3a-2 to 3a-4 to form liquid attachment areas 2-2 to 2-4. Fig. 10 shows the state when a liquid has been ejected from the nozzle opening 3a-4. Further ejection is performed as necessary to 3a-n to form attachment areas for different probes to 2-n. Further repetition of such operations enables continuous formation of a large number of probe carriers of the same configuration.

[0077]

Meanwhile, when a carrier is provided as a piece of a certain rigidity, application of a liquid to it using a liquid ejection apparatus is enabled by conveying the piece by a suitable support member such as a belt conveyer.

[0078]

When a mode is employed in which binding reaction between the probe and the substrate occurs on the substrate, the liquid containing the probe is applied to the substrate to form an attachment area. In this state, the probe starts to react with the carrier, whereby a probe immobilization area is formed there. While when a specific treatment for immobilizing the probe to the substrate is required, the substrate on which the probe liquid was attached is subjected to appropriate immobilization treatment.

[0079]

A carrier of thread, string, or belt form, the carrier may be fed to the liquid ejection apparatus as a bobbin or roll wound around a core. Such a carrier may be suitably fed to the liquid ejection apparatus by continuously unwinding by moving means in combination of a roller, guide and the like. An example of such a form is shown in Fig. 10. A thread-shaped carrier 1, provided as a roll, moves along a moving path with a roller pair, a guide roller and the like to the position where the liquid ejecting apparatus 3 is placed where a liquid containing a probe is applied to the carrier. Unwinding from the roll can be done by rotating the roller pair with suitable driving means. In that case, applying a suitable backward tension to the

roll can prevent the substrate from slacking. Preferably, the roll may include a dummy portion at the leading end thereof such that the dummy portion of the predetermined length passes through the installation section of the liquid ejection apparatus before the carrier part is fed into the apparatus.

[0080]

A cassette housing carrier rolls therein is a suitable mode for applying probe to a carrier unwound from a roll using a liquid ejection apparatus and recovering the resulting probe carrier as a roll. Fig. 11 schematically shows a cassette having such a configuration placed in a manufacturing apparatus provided with a liquid ejection apparatus. This cassette has a cabinet 4 incorporating a member 5 and a take-up reel 6 for housing a roll-shaped carrier together with various kinds of guide rollers. A roll-shaped carrier 1 moves by being wound around the reel 6 by rotational drive of the driving roller on the side of the manufacturing apparatus and of the reel 6. A probe solution is applied to this carrier at the opening 8 of the cabinet 4 from the liquid ejection apparatus 3. The part to which a probe solution was applied may be subjected to a drying process or a probe-immobilizing process before wounded to the reel 6, as required. Such processes may be applied via another opening provided in the

cabinet 4 from outside the cassette.

[0081]

A probe carrier wound to the reel 6 may be used in various forms. For example, the probe carrier may be taken out as a roll from the cassette to be shipped as a product; or may be unwound from the roll and cut at the predetermined length. As shown in Fig. 12, a plurality of spot arrangements of Fig. 6 may be made in parallel. This may be processed into the Fig. 6 spot arrangement by cutting the roll that has been wound onto the reel 6. Further, the cassette may be used as a supply opening in analytical procedures.

[0082]

It is necessary that the probe information (genes or proteins such as antibody) corresponds with the address on the tape-shaped carrier configured in a cassette. For address, by forming a magnetic recording layer on one surface of the tape, it is possible to store magnetic data information in this magnetic recording layer. Alternatively, information on the substance immobilized by knowing the elapsed time when the tape is scanned at a certain speed.

[0083]

Shown below is an example of an long probe carrier having the above-described configuration.

[0084]

Example 2

Fabrication of a plate for quantification of gene expression

A roll of cellulose-nitrate paper was set in an ink jet printer in the same manner as paper. Six kinds of oligonucleotide probe (DNA) having different sequences were filled into six ink cartridges of this ink jet printer and printed on the cellulose-nitrate paper as shown in Fig. 10.

[0085]

Specifically, 1 μmole solutions of different probes were filled separately in the liquid container of the liquid ejection apparatus and the cellulose-nitrate paper was moved along the alignment line of nozzle openings of the liquid ejection apparatus, whereby respective solutions were attached to the predetermined part. A probe carrier was thus obtained having areas where different oligonucleotides are immobilized in the longitudinal direction.

[0086]

Then the nitrocellulose paper was wound again after probe printing as shown in Fig. 11, and the probe DNA was immobilized by heating the paper to 180°C. Subsequently, the paper roll was cut to fabricate a belt-like sheet having a pattern as shown in Fig. 6.

[0087]

<Analysis of expression amount using a tape for quantification of gene expression>

Reaction between a target substance contained in a sample and a probe immobilized on a carrier can be signalized by various methods. Usually a labeling substance is used, such as a fluorescent substance that can emit optical signal detectable by a sensor and the like.

[0088]

mRNA amplification and fluorescent-labeling methods are carried out by the conventional in vitro transcript method (e.g., the MegaScript kit, Ambion Ltd.), as in Example 1.

[0089]

When such a sample solution is contacted with the probe carrier and then the reaction between the fluorescent labeled target substance and the probe is detected as fluorescence, the presence or absence of reaction between the probe and the target substance in each probe immobilization area can be determined by moving the long probe carrier or the detection means such as a sensor.

[0090]

Further, the roll housing part of the cassette shown in Fig. 11 can be set with a sample roll for measurement, i.e., a probe carrier contacted with a sample solution and in a state suitable for optical

detection of the presence or absence of reaction between the probe and the target substance. Carrying out a winding operating with a winding reel 6 at the timing required for measurement, it is possible to optically measure the presence or absence of a reaction by sensing means, such as a sensor, at the opening 8. At this time, in the case of the array having an arrangement as shown in Fig. 4, simple and convenient measurement of signal intensity is also possible by measuring the distance between the signal generating point and the point where signal disappears completely and calculating from the distance information the signal intensity as an area.

[0091]

The detection apparatus that is used with a cassette may include a fitting part for a cassette having within a cabinet the aforementioned housing part for housing a sample roll wound to a feed reel, a take-up reel for taking up the sample roll fed from the feed reel and an opening provided along the moving path of the measurement sample extending between the roll housing part and the take-up reel; driving means for driving the take-up reel of the cassette arranged in the fitting part; and aligning means for aligning the signal detection means of the detection apparatus to a position where it can detect the signal from a probe immobilization area of the

measurement sample that is driven to move on the moving path by the driving means via the opening.

[0092]

Although the above-described examples illustrate embodiments when a line sensor is used, the present invention is not limited to this configuration. When local sensors, such as an area sensor, are used, different probes can be disposed in the end side direction.

[0093]

[Effect of the Invention]

As described above, according to the present invention, there can be provided the analysis method of simultaneously and readily performing quantitative analysis on a plurality of different kinds of target substances in a solution.

[0094]

Specifically, even in a system in which DNAs which are large and small in expression amount are mixedly present, the probe DNA of the mount exceeding the amount of each DNA is immobilized onto the carrier, so that there can be provided the method of simultaneously performing quantitative analysis on all the DNAs.

[Brief Description of the Drawings]

[Figure 1]

A diagram illustrating one embodiment of the

probe carrier in accordance with the present invention where the number of spots varies with the kinds of probes immobilized.

[Figure 2]

A diagram illustrating another embodiment of the probe carrier in accordance with the present invention where the carrier is divided to a plurality of areas, where the same kind of probe molecules are immobilized as a plurality of spots in respective areas and the number of spots varies with the kinds of the probes.

[Figure 3]

A diagram illustrating the other embodiment of the probe carrier in accordance with the present invention wherein the carrier is divided into a plurality of areas, and the same kind of probe molecules are immobilized in each area and each area contains the same number of spots but the number of probe molecules per spot varies with the kinds of the probes.

[Figure 4]

A diagram illustrating another embodiment of the probe carrier in accordance with the present invention.

[Figure 5]

A diagram illustrating another embodiment of the probe carrier in accordance with the present

invention.

[Figure 6]

A diagram illustrating another embodiment of the probe carrier in accordance with the present invention.

[Figure 7]

A diagram illustrating another embodiment of the probe carrier in accordance with the present invention.

[Figure 8]

A diagram illustrating another embodiment of the probe carrier in accordance with the present invention.

[Figure 9]

A diagram illustrating another embodiment of the probe carrier in accordance with the present invention and the use of the probe carrier.

[Figure 10]

A schematic diagram illustrating the manufacturing method of the probe carrier in accordance with the present invention.

[Figure 11]

A schematic diagram illustrating the configuration of a cassette having the probe carrier in accordance with the present invention.

[Figure 12]

A diagram illustrating a further embodiment of

the probe carrier in accordance with the present invention.

[Description of Reference Numerals or Symbols]

1: carrier

2: probe immobilized area

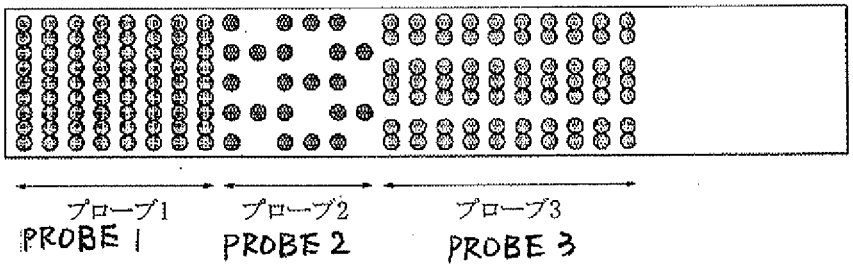
3: liquid ejecting apparatus

4: cabinet

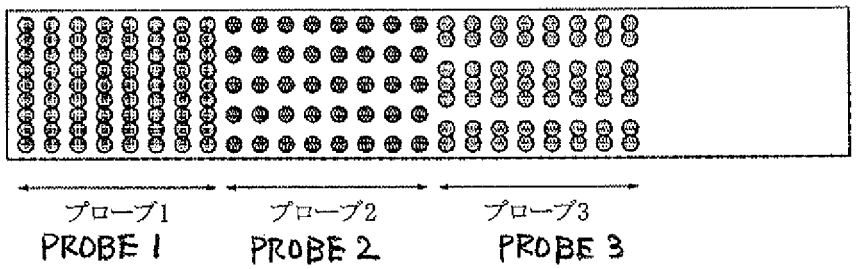
5: carrier containing member

6: take-up reel

【書類名】　図面
[Name of the Document] Drawings
【図１】
[Fig. 1]



【図２】 [Fig. 2]



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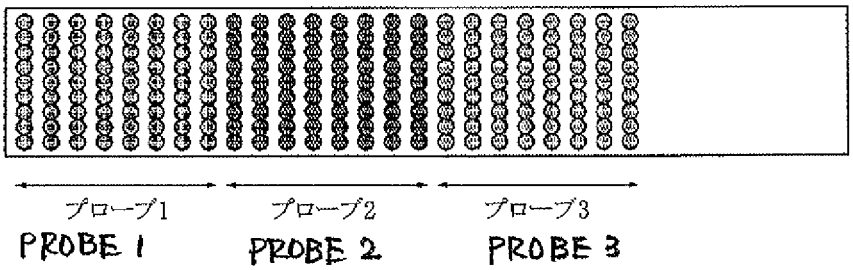

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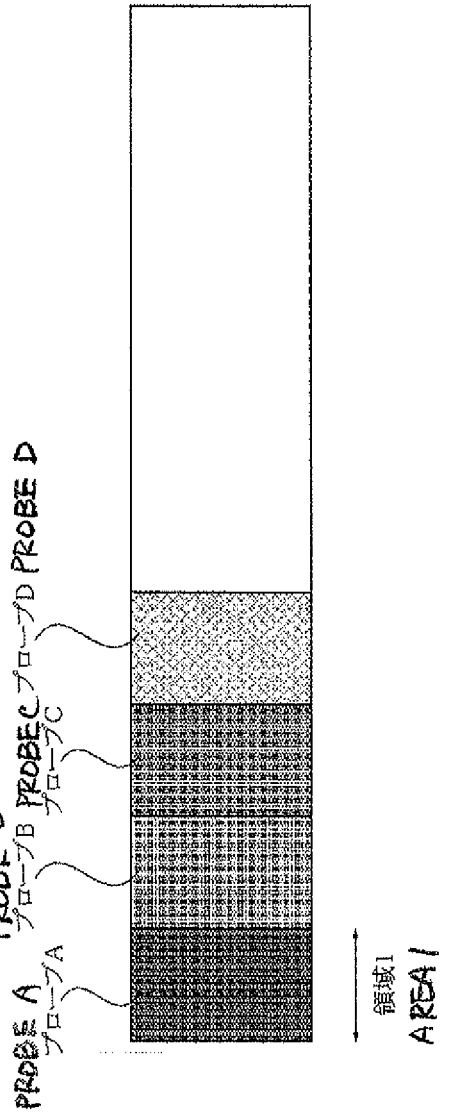
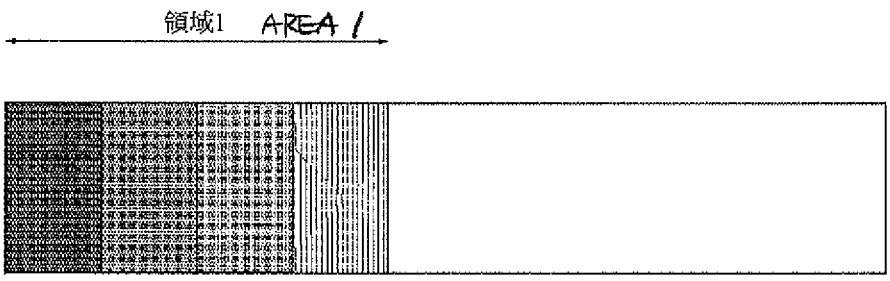
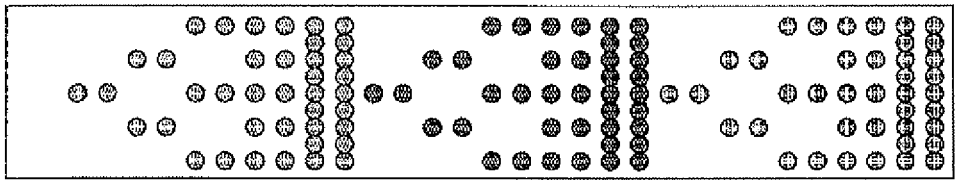

PROBE A
プローブA
PROBE B
プローブB
PROBE C
プローブC
PROBE D
プローブD
領域1
AREA 1

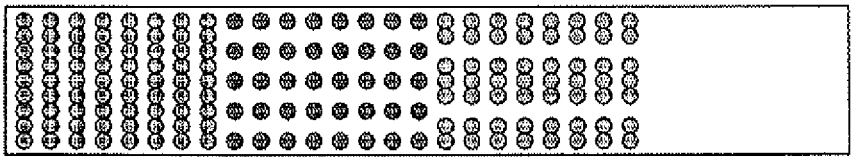
【図５】 [Fig. 5]



【図６】 [Fig. 6]

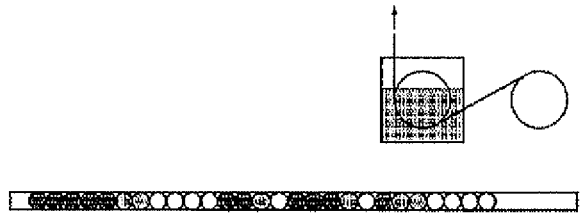


【図７】 [Fig. 7]

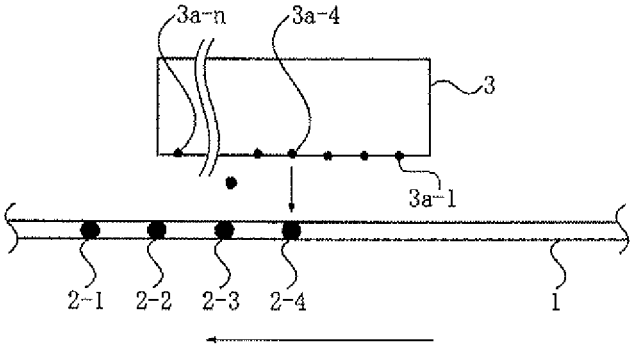


【図８】 [Fig. 8]

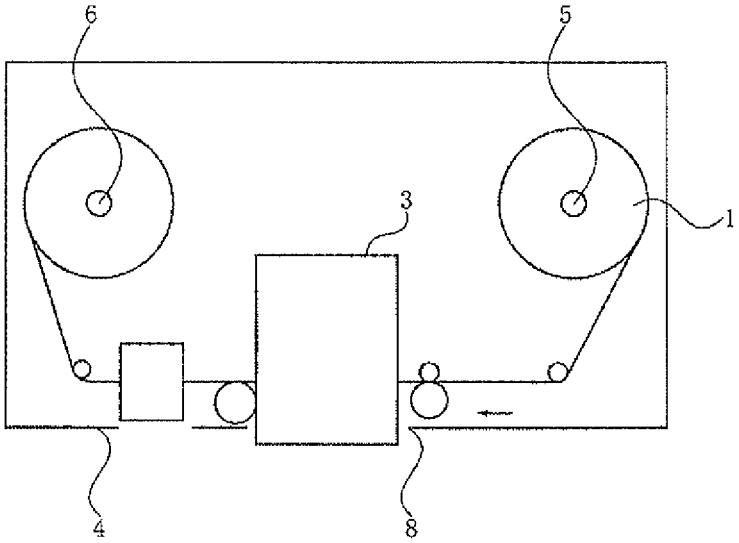
【図９】 [Fig.9]


反応済み
未反応
ALREADY SUBJECTED TO REACTION
NOT YET SUBJECTED TO REACTION

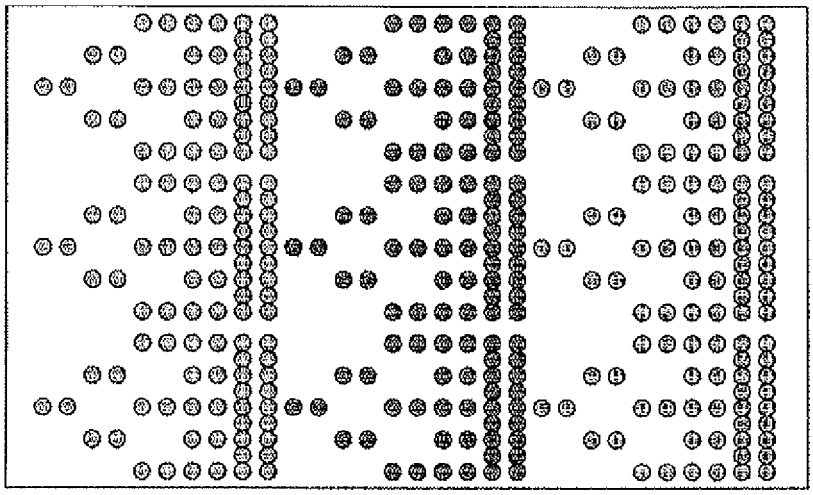
【図１０】 [F: 7.10]



【図１１】[Fig. 11]



【図１２】[Fig.12]



[Name of the Document] Abstract

[Abstract]

[Subject] An object of the present invention is to provide a method of simultaneously and readily quantifying a plurality of target substances in a solution.

[Solving Means] A quantifying method comprising the steps of preparing a probe carrier in which probes which can be specifically bonded to the target substances are immobilized at known positions on the carrier, contacting the carrier with the solution to bind the target substance to the probes; and measuring the amount of the target substance bonded to the probe, wherein the probes which can be bonded to the target substance of the amount exceeding the amount of each of the target substances in the solution are immobilized onto the probe carrier.

[Elected Drawing] Figure 1